BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

THIS PAGE BLANK (USPTO)

(12) UK Patent Application (19) GB (11) 2 248 688 (13) A

(43) Date of A publication 15.04.1992

(21) Application No 9111965.1

(22) Date of filing 04.06.1991

(30) Priority data
(31) 2027434 (32) 12.10.1990 (33) CA

(51) INT CL[5]
G01N 33/68 33/543

(52) UK CL (Edition K)
G1B BAE BAG B103 B309 B402 B504 B523
U1S S1059 S1337

(56) Documents cited
WO 91/01498 A
European Heart Journal, Vol.8, 1987, pages 989-994.
Scan. J. Clin. Lab. Invest., Vol.44, 1984, pages 679-682.

(58) Field of search
UK CL (Edition K) G1B BAD BAE BAG
INT CL[5] G01N
DIALOG: WPI; BIOTECH

(71) Applicant
George Jackowski
RR #3 1st Line North, Halton Hills, Ontario L9T 2XY, Canada

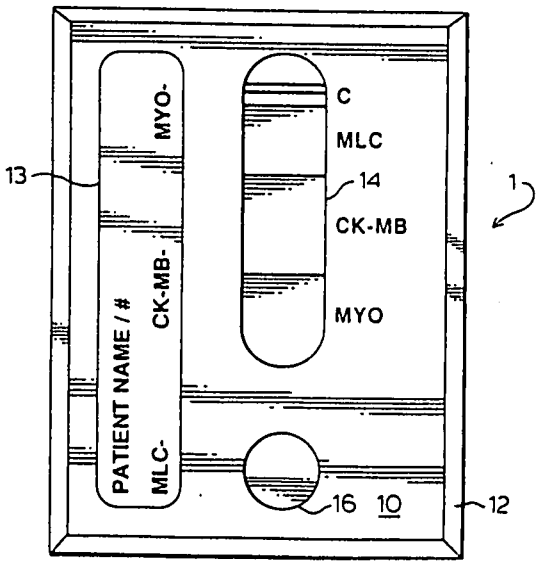
(72) Inventor
George Jackowski

(74) Agent and/or Address for Service
D Young & Co
10 Staple Inn, London, WC1V 7RD, United Kingdom

(54) Diagnosing myocardial infarction

(57) A diagnostic kit is disclosed for differentiating myocardial infarction at early onset of patient chest pain. The test kit is preferably in panel form (1) and comprises a receptacle (16) for receiving and retaining a sample of blood or serum of the patient and at least three monoclonal or polyclonal antibodies suspended on a carrier and visible through a window (14). Each antibody is complementary to a different protein released by the heart muscle during early stages of a myocardial infarction and the kit comprises corresponding reagents in dry chemical form such that the combined response of reagents visible through the window (14) indicates the diagnostic condition of the patient. Suitable antibodies are those complementary to creatine kinase, myoglobin, myosin light chains, troponin, sarcolemma membrane proteins, triose P isomerase, tropomyosin.

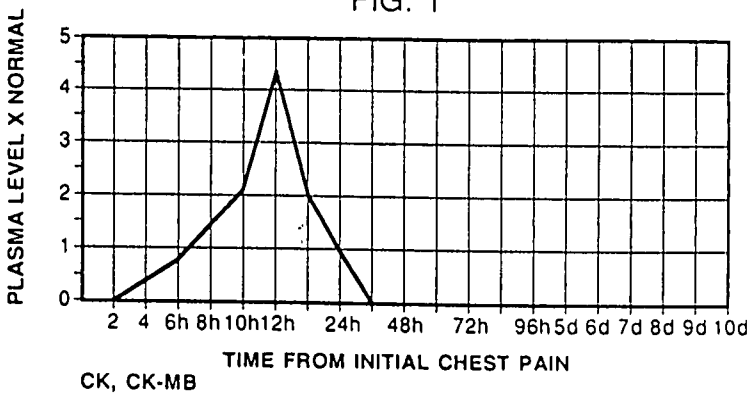
FIG. 8



GB 2 248 688 A

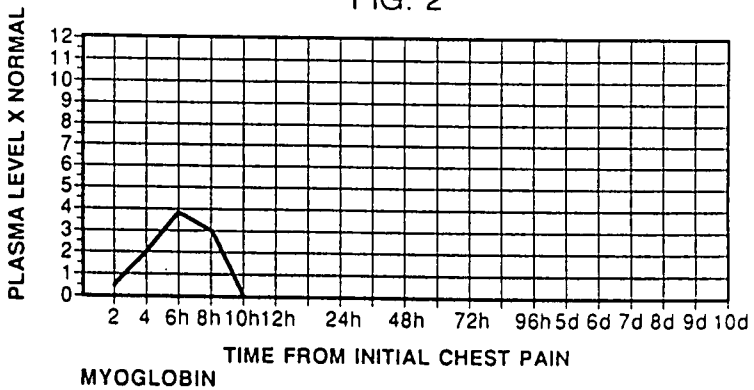
THIS PAGE BLANK (USPTO)

FIG. 1



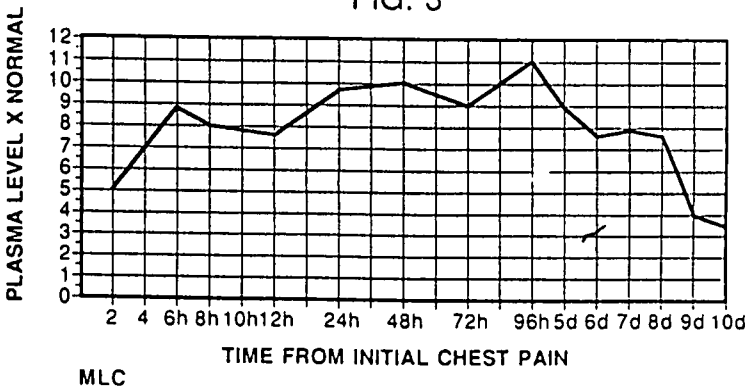
CK, CK-MB

FIG. 2



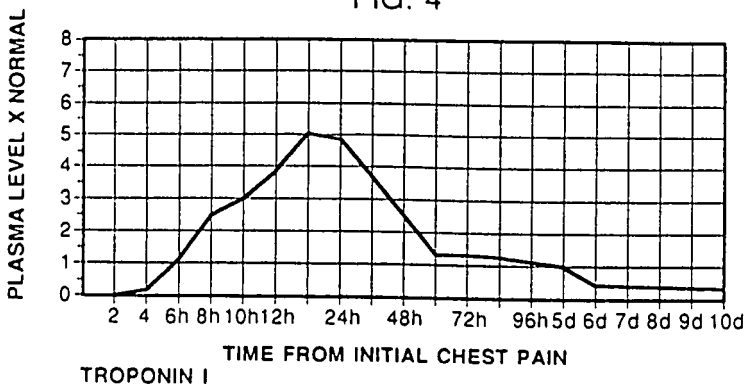
MYOGLOBIN

FIG. 3



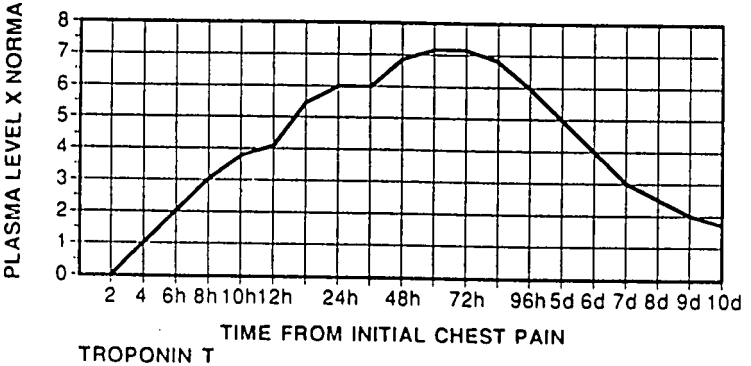
MLC

FIG. 4



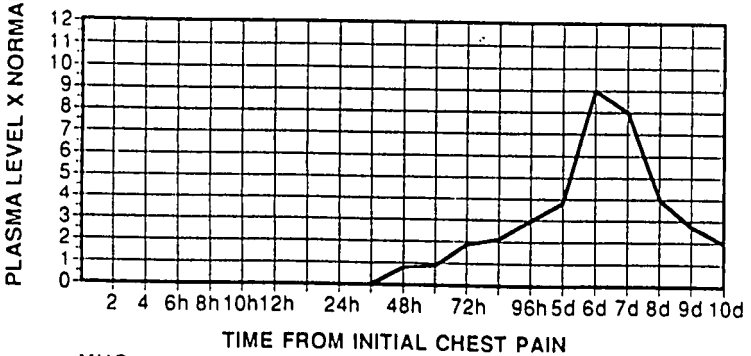
TROPONIN I

FIG. 5



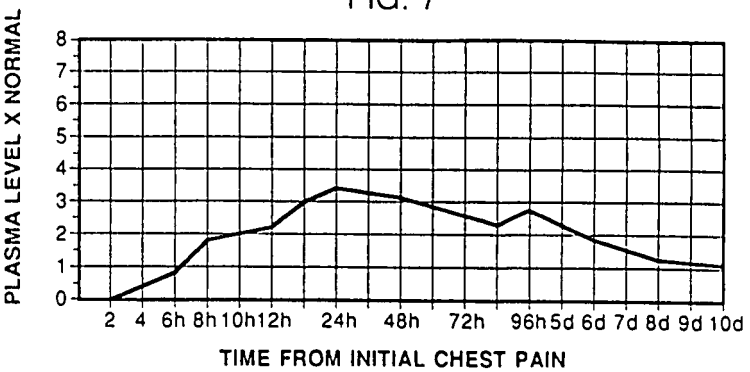
TROPONIN T

FIG. 6



MHC

FIG. 7



TROPOMYOSIN

FIG. 8

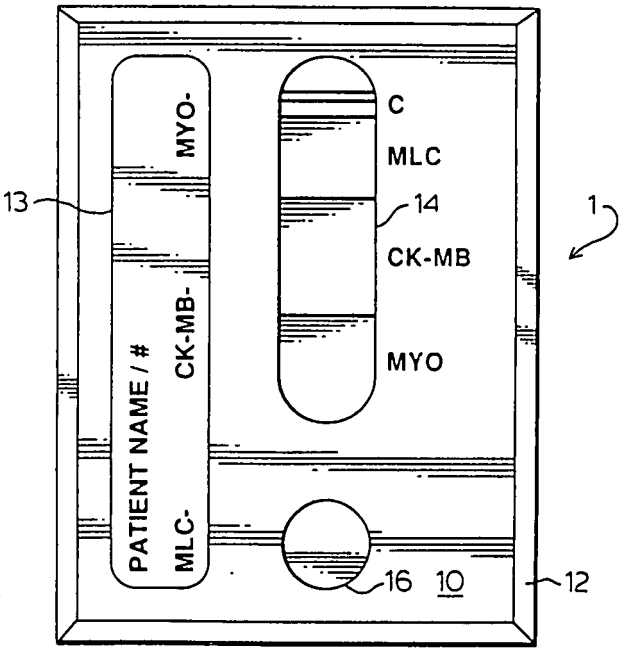


FIG. 9

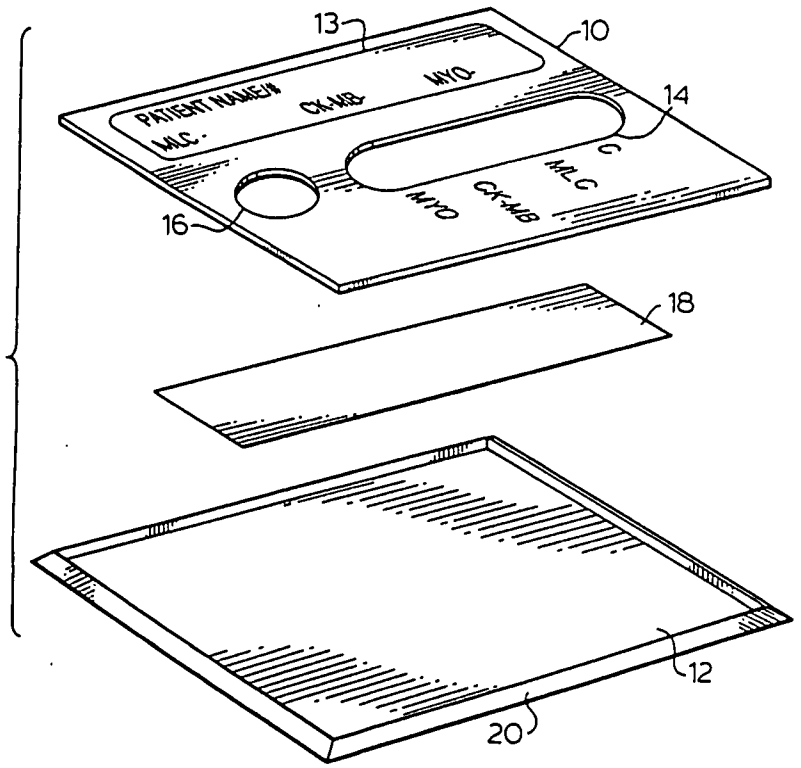


FIG. 10

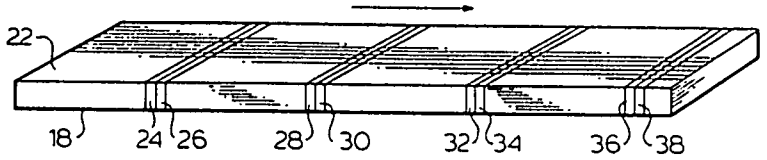
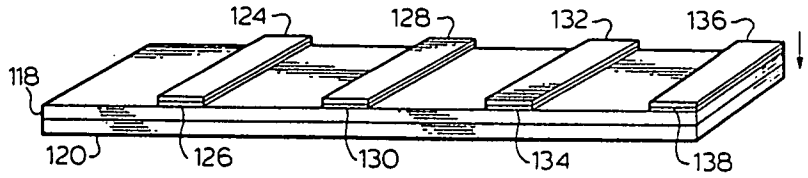


FIG. 11



DIAGNOSTIC KIT FOR DIAGNOSING AND DISTINGUISHING CHEST PAIN IN EARLY ONSET THEREOF

This invention relates to a diagnostic kit for providing an accurate, simple and rapid differential diagnosis as between unstable angina and myocardial infarction ("MI") at the early onset of patient chest pain.

Emergency diagnosis of myocardial infarction largely depends on physician acuity and assessment of a patient's symptoms, such as chest pains or pressure, possibly radiating down the arm and up the neck, fatigue, sense of impending doom, shortness of breath, pallor, cold clammy skin, peripheral cyanosis or rapid thready pulse.

Most North American patients experiencing chest pain will report to a doctor or emergency room within six (6) hours after the onset of the chest pain. It is therefore essential that a diagnostic test be effective in the early stages of an MI.

Several cardiac tests have been used to detect MI. These tests include: ECG, SGOT/AST, LDK, CK-MB Immunoassay and NA Latex Myoglobin Particle Enhanced Assay. However, there are no single enzyme cardiac test which enable the emergency department physician to identify the source of chest pain as cardiac or non-cardiac. Further, it is only after a myocardial infarction has been confirmed that thrombolytic therapy may be initiated. However, the earlier such therapy is initiated, the greater likelihood of full recovery of the patient or at least minimization of cardiac damage. It is therefore essential for a physician to decide as soon as possible whether chest pains are cardiac or non cardiac in origin.

The electrocardiogram (ECG) may be used to detect an MI. However

ECG is not diagnostic until after the heart has suffered severe damage. The diagnostic specificity of the ECG is only 51% in the initial phase of chest pain. Therefore, ECG is not suitable for early detection of MI.

Serum glutamic oxalacetic transaminase/aspartate transferase (SGOT/AST) is a predominant enzyme found in high concentration in heart muscle. Serum tests to determine levels of SGOT are used in diagnosing myocardial infarction. However, SGOT only begins to rise about 8-10 hours following the onset of chest pain, peaks within 24-36 hours and returns to normal after 5-7 days. SGOT is not particularly helpful in diagnosing myocardial infarction in an emergency setting at an early stage of patient chest pain. Also, SGOT is not specific to cardiac muscle. It is found in many tissues including skeletal muscle, liver and kidney, being released as a result of intro muscular injections, shock, during liver disease, and hepatic congestion, and is therefore of little value in detecting specific cardiac tissue injury.

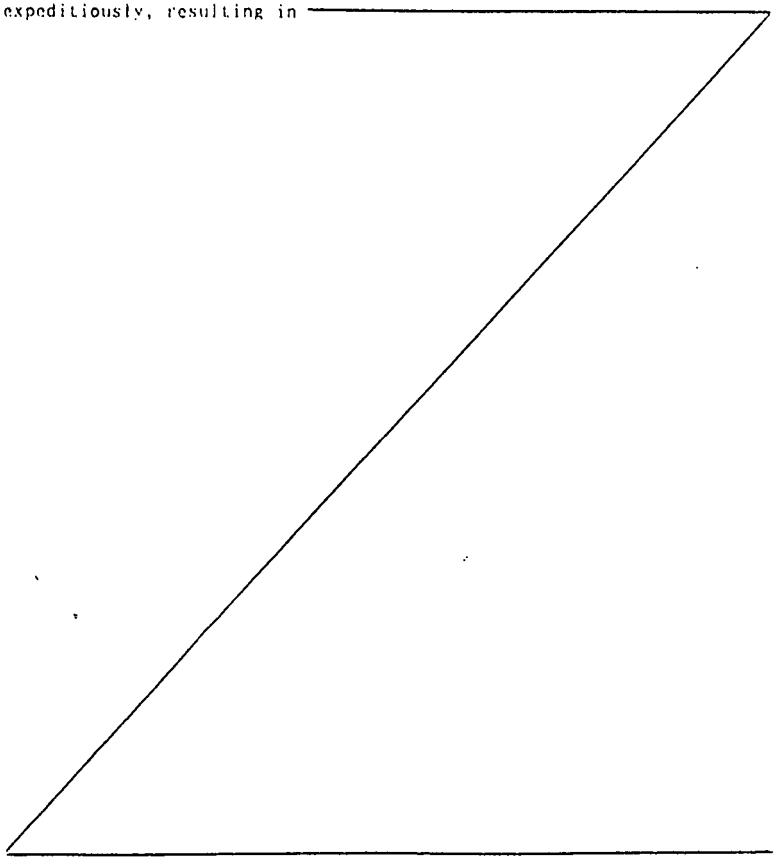
Lactate Dehydrogenase (LDH) is an enzyme found in high concentration in many tissues, including heart, skeletal muscle and liver. Tests to detect the presence of LDH in serum are used to diagnose myocardial infarction. There are five common isotypes of which the heart contains predominantly LDH1 and LDH2. LDH levels begin to rise 24-36 hours after the onset of chest pain, and peak after 48-72 hours, returning to normal after 4-8 days. LDH is therefore not useful as an indicia of MI at an early stage of patient chest pain. In addition, LDH is not specific to cardiac damage, and appears with pulmonary embolism, haemolysis, hepatic congestion, renal disease and skeletal muscle damage. This lack of specificity also decreases the utility of LDH as a diagnostic aid.

Creatine kinase (CK) is an enzyme found in muscle tissue. CK catalyses the conversion of creatine and adenosine triphosphate (ATP) to phosphocreatine and adenosine diphosphate (ADP). One of several CK isoenzymes is CK-MB which is found in cardiac tissue. CK-MB is a sensitive marker for the detection of myocardial infarction, as it is released from damaged myocardium tissue. CK-MB thereafter present in the serum of an affected individual. Figure 1 illustrates the concentration of CK in the serum of a patient as a function of time. (ref. Lee T.H. et al. (1986) Ann. Intern. Med. 105, 221-233).

The CK-MB immunoassay is the standard diagnostic test for myocardial infarction. A method describing the use of CK-MB is disclosed in U.S. Patent No. 4,900,662 entitled "CK-MM Myocardial Infarction Immunoassay". This method involves determining the initial elevated concentration level of CK-MM-a, an isoform of CK-MM, and CK-MM-a and CK-MM-b concurrently in patient serum following a myocardial infarction. Use of the method provides an accurate estimation of the time of the infarction. The method involves determining the combined concentration of CK-MM-a and CK-MM-b and the concentration of CK-MM-a in serum, in order to determine the time of the acute phase of myocardial infarction. Reagents are disclosed and comprise novel polyclonal and monoclonal antibodies for CK-MM-a which do not bind significantly with CK-MB, CK-MM-b or CK-MM-c, an anti-CK-MM-b antibody which does not bind significantly with CK-MB, CK-MM-a or CK-MM-c, an anti-CK-MM-a+b antibody which binds with CK-MM-a and CK-MM-b but does not bind significantly with CK-MB or CK-MM-c, labelled derivatives of these antibodies, insoluble supports to which these antibodies are adhered, and kits containing one or more of these reagents. Enzyme labelled and radiolabelled CK reagents are particularly useful.

There are difficulties with the use of CK-MB alone as a diagnostic marker. First, serum levels of CK-MB are not elevated until 6 - 8 hours after the onset of myocardial infarction, and do not peak until after 12 hours, making early emergency diagnostic and treatment difficult.

Secondly, the CK-MB test must be conducted in a laboratory by trained laboratory technicians. In non-urban locations, it may not be feasible to have the test conducted and the results interpreted expeditiously, resulting in



increased delay in diagnosis and hence increased costs to the health care system in terms of hospitalization costs of a patient awaiting diagnosis.

Thirdly, CK-MB has been located in normal skeletal muscle tissue, consequently rendering the test less cardiac specific, and the diagnosis less certain.

Myoglobin is another protein located near the skeletal or myocardial cell membrane. It is expelled from the cell as soon as the cell membrane becomes abnormally permeable, for example, during myocardial ischemia, a reversible state. Myoglobin is detectable in the serum within 1.5 hours of the onset of chest pain. The medical research community believes that myoglobin is released by myocardial necrosis, and it is therefore a useful early marker of myocardial injury. Figure 2 illustrates the concentration of myoglobin in the serum as a function of time. (ref. Grenadier E. et al. (1981) Am. Heart J. 105, 408-416; Seguin J. et al. (1988) J. Thorac. Cardiovasc. Surg. 95, 294-297)

In determining the origin of chest pain, an acute myocardial infarction can be excluded if no elevation of serum myoglobin is detected within 2 - 3 hours after the onset of pain.

An NA Latex Myoglobin Particle Enhanced Assay is a commercially available assay kit for the detection of myoglobin. The assay is based on the reaction between antigen present in human body fluids and antimyoglobin antibodies covalently coupled to polystyrene particles. The sample, N Myoglobin Reagent, a solution for the elimination of nonspecific reactions and N Reaction Buffer are pipetted

automatically into a cuvette. Light scattering is measured by a nephelometric procedure after 12 minutes of incubation time and the myoglobin concentration is calculated from a calibration curve.

Myoglobin may also be assayed using a radioimmunoassay but there is no enzyme-linked immunosorbent assay (ELISA) format yet available.

There are difficulties with the use of myoglobin alone as a diagnostic marker. Myoglobin does not indicate a particular type of myocardial injury, such as myocardial infarction. Myoglobin can also be present during such diverse conditions as shock, renal disease, rhabdomyolysis, and myopathies. Additionally, myoglobin concentrations in serum and plasma generally depend on age and sex and vary over a wide range in normal healthy humans. Serum concentrations up to 90 ug/l are generally regarded as the upper limit of the reference range for healthy people. Therefore, what may be a normal level for one individual may be indicative of a serious problem in another individual, making diagnosis somewhat less accurate than would be desirable.

Myosin light chains (MLC) are integral parts of the myosin myofibril, but their functional role is still unclear. MLCs exist in slow, fast, atrial, and ventricular muscles. It is known that MLCs are highly sensitive for myocardial ischemia. MLCs appear in the serum rapidly, and their levels remain elevated for up to 10 days following myocardial necrosis. Figure 3 illustrates the concentration of MLC in patient serum as a function of time. (ref. Wang J. et al. (1989) Clin. Chimica. Acta 181, 325-336; Jackowski G., Symmes

J. C. et al. (1989) Circulation Suppl. 11 80, 355.) MLC also has prognostic value in determining the success of thrombolytic therapy. Higher levels of MLC, indicate a worse prognosis, and also corresponds to a larger infarction. Falling levels over several days indicate a tendency towards patient recovery, whereas spiking or stadico pattern indicate a tendency towards infarction and a need for intervention.

There are two principal types of MLC known as MLC1 and MLC2, which exist as a soluble pool in the myocardial cell cytoplasm and also integral with the myosin myofibril. In the ventricular muscle, MLC2, and perhaps MLC1, is identical with the isotype expressed in slow skeletal muscle. MLC1 is elevated in 80-85% of the patients with cardiac pain. MLC1 is a very sensitive indicator of unstable angina and coronary heart disease.

Other cardiac markers, low molecular weight cardiac proteins (LMWCP) may be used as cardiac markers. Examples of such cardiac markers include components of the contractile apparatus, namely, troponin, troponin-T, troponin-I and troponin C, mitochondrial enzymes, such as triose P isomerase, low molecular weight polypeptides which are readily released from the heart, and sarcolemmal membrane proteins or protein fragments which may be released early after the onset of ischemia, in particular, a 15kd sarcolemma protein and a 100kd complex glycoprotein which are cardiac specific.

The cardiac isotype troponin-I inhibits the interaction between actin and myosin molecules during rest periods between contractions of the heart muscle. Troponin-I appears in serum of patient within 4-6 hours after MI and remains elevated for 7

- 8 days. Figure 4 illustrates the concentration of troponin-I as a function of time. (ref. Cummins B., Auckland M.L. and Cummins P. (1987) Am. Heart J. 113, 1333-1344.) It is cardiac specific and has a greater sensitivity than other markers in detecting cardiac versus skeletal muscle injury.

Troponin-T is part of the troponin-tropomyosin complex of the thin filament and serves as a link between the tropomyosin backbone and the troponin-I troponin C complex. Troponin-T is a basic protein and has isotypes in cardiac and fast and slow skeletal muscles. It appears in serum within 3 hours and remains elevated for at least 10 days following MI. Figure 5 illustrates the concentration of troponin-T as a function of time. (ref. Katus H.A. et al. (1989) J. Mol. Cell Cardiol. 21, 1349-1353.) Troponin-T follows a biphasic release pattern. It is cardiac specific and very sensitive for MI.

Myosin heavy chains (MHC), and tropomyosin, are heavier molecular weight proteins which may also be used as cardiac markers. MHC is part of the major contractile protein of muscle. Fragments of MHC can be released from the ventricule into serum after myocardial cell necrosis and subsequent irreversible membrane injury. Although MHC fragments do not appear quickly in the serum following myocardial cell necrosis, MHCs remain elevated for at least 10 days following MI, and peak levels of MHC are observed 4 days after MI. Figure 6 illustrates the concentration of MLC as a function of time. (ref. Leger J.O.C. et al. (1985) Eur. J. of Clin. Invet. 15, 422-429, Seguin J.R. et al. (1989) J Thorac. Cardiovasc. Surg. 3, 397-401.) The area under the MHC release curve correlates

very well with the extent of myocardial cell damage. However, MHC levels are of little clinical value during the acute phase of MI.

Tropomysin is a dimer formed from two polypeptides which are part of the regulatory system in muscle contraction. Tropomyosin is detectable in serum approximately 7-8 hours after myocardial infarction, and like CK-MB, is very sensitive for myocardial infarction. Figure 7 illustrates the concentration of MLC as a function of time. (ref. Cummins P. et al. (1981) Clin. Sci. 60, 251-259). However, tropomyosin is not cardiac specific since it is elevated in conditions of skeletal muscle trauma.

There are limitations for each of the current standard diagnostic methods for myocardial infarction. None provide a highly sensitive, specific, rapid, and simple diagnostic test which may be conducted soon after the onset of chest pain, for example, in an ambulance or doctor's office.

The present invention resides in a diagnostic kit comprising the necessary enzyme immunoassay components preferably in dry chemical format to enable the measurement of three different markers of cardiac damage present in the blood or serum of a patient and which can be used in emergency settings to determine whether the patient is suffering from unstable angina or whether a myocardial infarction has taken place, even up to several days following the onset of pain. Moreover, serial temporal measurements with the kit will offer prognostic information to the physician as to the extent of muscle damage and the success of thrombolytic intervention. In the preferred embodiment of the invention, the three markers are creatine kinase (CK), myoglobin, and myosin light chains (MLC).

In accordance with the present invention therefore there is

provided a diagnostic kit for detecting a myocardial infarction at early onset of patient chest pain, comprising:

i) a receptacle for receiving and retaining a sample of blood or serum of the patient, and

(ii) a detection means associated with the receptacle and comprising:

a) three or more monoclonal or polyclonal antibodies supported on a solid carrier, each antibody being complementary to a different protein released by the heart muscle during early stages of a myocardial infarction and contactable by the sample, and

b) the necessary reagents independently responsive to each antibody when reacting with its complementary protein, and which collectively provide a response indicative of the diagnostic cardiac condition of the patient.

Preferably said receptacle and said detection means form an integral structure containing said immobilised antibodies and said reagents in dry chemical form, and preferably said antibodies are complementary to at least three of the following proteins or enzymes: creatine kinase, myoglobin, myosin light chains, troponin, troponin-I, troponin C, troponin-T and sarcolemmal membrane proteins, triose P isomerase or any low molecular weight cardiac proteins having the characteristics and properties of creatine kinase, myoglobin or myosin light chains, tropomyosin or any heavy molecular weight cardiac proteins having the characteristics and properties of creatine kinase, myoglobin or myosin light chains, wherein at least two are selected from creatine kinase, myoglobin, myosin light chains and troponin-T.

The invention will be further described with reference to the

accompanying drawings, in which:

Figure 1 is a graph illustrating the level of CK in serum as a function of time;

Figure 2 is a graph illustrating the level of myoglobin in serum as a function of time;

Figure 3 is a graph illustrating the level of MLC in serum as a function of time;

Figure 4 is a graph illustrating the level of troponin-I in serum as a function of time;

Figure 5 is a graph illustrating the level of troponin-T in serum as a function of time;

Figure 6 is a graph illustrating the level of MHC in serum as a function of time;

Figure 7 is a graph illustrating the level of tropomyosin in serum as a function of time;

Figure 8 is a plan view of the preferred embodiment;

Figure 9 is an exploded perspective view of the embodiment of Figure 8;

Figure 10 is an oblique view of the membrane of the embodiment of Figure 8; and

Figure 11 is an oblique view of a second embodiment of the membrane.

Referring to the drawings the main component of the kit is generally illustrated in Figure 8 and comprises a dry format triple enzyme immunoassay in a panel format identified as 1.

The panel format to be used in known and is commercially available. The panel format is similar to a

format currently being used in association with pregnancy testing and is commercially available under the trade-mark BIOSIGN.

The panel consists of a polypropylene card having a front panel 10 and a back panel 12. Front panel 10 has a display window 14, one for each cardiac marker and a sample window 16, as illustrated in Fig. 1. Underneath front panel 10 is an exposed dry chemistry membrane 18 which is affixed to the back of front panel 10 by suitable means. Back panel 12 is provided with a lip 20 which extends around the perimeter of back panel 12 for receiving front panel 10 in a snap fit thereby sealing the membrane 18 between the front and back panel.

While the front and back panel have been described as being snapped together, there are numerous other suitable methods of joining the two together which would be apparent to a person skilled in the art.

Front panel 10 may also be provided with an area 13 upon which the patient's name or identification may be written. Also space may be available to write the results of the test.

With reference to figure 10, membrane 18 is the carrier of the monoclonal or polyclonal antibodies. In the preferred embodiment, the flow of blood or serum is from one end to the other end as shown by the arrow. End 22 is aligned with sample window 16. An immobilized captured antibody 24 is layered against or bonded to an antibody-enzyme conjugate 26 which is directed against a different epitope on the antigen than that which is recognized by the antibody 24. Antibody 24 is

complementary to the myosin protein. Similarly, antibody 28 is layered with a corresponding reagent 30. Antibody 28 is complementary to CK-MB. Likewise, antibody 32 is layered with a reagent 34. Antibody 32 is complementary with the myosin light chain. Antibody 36 is one which is complementary to any protein found in normal serum or blood. Antibody 36 is layered with reagent 38.

The monoclonal and polyclonal antibodies can be prepared by using conventional procedures with any mammal used for polyclonal antibody production.

In the preferred embodiment, a labelled reagent is used. The antibody reagent is labelled or chemically bonded to a distinctive moiety which can be observed or measured to verify or quantify the presence of an antibody in the serum or blood or on the dry chemistry membrane. Ligands and groups which can be conjugated to the antibodies of this invention for use as a diagnostic tool include elements, compounds or biological materials which have physical or chemical characteristics which can be used to distinguish the antibodies to which they are bonded from other antibodies.

At least two antibodies of the type mono/poly or rabbit/poly, goat/poly per cardiac marker are required. The antibodies are affinity purified against their specific cardiac immunogen and then further purified by cross-adsorption against a non-related species to eliminate non-specific immunoglobulins.

In use, the diagnostician, for example a physician, ambulance attendant or nurse, adds three drops or less than 100 ml of the patient's serum or blood to the sample window

16. The sample will migrate along the membrane 18 by capillary action and will successively come into contact with the antibody and reagent pairs 24 and 26, 28 and 30, 32 and 34 and 36 and 38.

The specific cardiac marker if present in the sample binds to the antibody immobilized on the membrane. The corresponding reagent will also react and is visualized by a change in colour of the reagent. The colour change is proportional to the concentration of the marker in the sample. Therefore if the test kit is used in timed intervals the increase or decrease in marker concentration can also be determined and used as a diagnostic tool. The results of the test should be completed within 3 - 5 minutes.

In the preferred embodiment, a blue band will show for each cardiac marker which is present in the sample. The intensity of the band is quantifiable using a reflectometer, which relates the colour intensity to the concentration level of a particular marker. The reflectometer may contain a micro-processor, so that the quantified result for each cardiac marker being tested in the panel may be produced and printed out as a concentration of each marker along with the patient's name or identification.

The test preferably is sensitive to marker concentrations from .5ng/ml to 25ng/ml using 3 drops or less than 100ul of serum or plasma with a within run and between run precision coefficient of variation of less than 15%.

The cardiac markers utilized in the test will depend on the properties of those markers. In the preferred embodiment, there will be a panel having myoglobin, MLC, and CK-MB, as illustrated in Figure 8.

Myoglobin is released very early from the myocardial cell, is not cardiac specific, has a very high sensitivity for myocardial infarction and necrosis and is not released by anoxic injury in the absence of necrosis. MLC is cardiac specific, and permits differentiation of cardiac from non cardiac pain, and is released early but not as early as myoglobin. CK-MB differentiates angina from myocardial infarction, but is not detectable until approximately six hours after the onset of chest pain and therefore is not of use alone as an emergency diagnostic test.

Referring to figures 1, 2 and 3 and if the three cardiac markers to be used are CK-MB, myoglobin, and MLC, the following interpretation of the results would provide a diagnosis.

If the panel shows positive for MLC and negative for myoglobin and CK-MB, it would indicate that the patient's chest pain is cardiac and that the source is unstable angina.

If myoglobin and MLC are positive and CK-MB is negative it would indicate an early evolving myocardial infarction and intervention therapy could be initiated.

If all three are positive, it would indicate a myocardial infarction.

If MLC and CK-MB are positive and myoglobin is negative, it would indicate a myocardial infarction.

If myoglobin and CK-MB are positive and MLC is negative, the patient could have skeletal muscle trauma (a false positive) or be in the midst of a myocardial infarction.

The test could not distinguish between a false positive and a "small" myocardial infarction in this case, as the MLC release curve has slight dips at several intervals and the patient may have a small subendocardial infarction and be tested at the time of a "dip". When the infarct is small, the "dip" is down to almost normal levels, and therefore the patient would test negative for MLC. Positive diagnosis would rely on the presence of CK-MB.

In the event that the patient is having a large myocardial infarction, the "dip" in MLC levels will not be so large as to be the same as normal levels, and therefore, MLC will remain detectable.

In other embodiments, the test panel may utilize different combinations of antibodies in the same format, such that different cardiac markers are assessed. In order to ensure that the panel will detect cardiac tissue damage at an early stage of patient chest pain, it is necessary to utilize at least one antibody corresponding to a marker which is present in large quantities at an early stage of cardiac damage, such as CK, myosin light chains or myoglobin. Low molecular weight cardiac proteins having the characteristics and properties of CK, myosin light chains or myoglobin may also be used in the kit.

Suitable proteins and enzymes may be selected from the folowing: troponin, troponin-I, troponin C, troponin-T and sarcolemmal membrane proteins, triose P isomerase or any heavy

molecular weight cardiac proteins having the characteristics and properties of creatine kinase, myoglobin or myosin light chains.

Other proteins such as tropomyosin, and myosin heavy chains may also be added to the kit. The kit would then be able to detect MI if the patient arrives for diagnosis many hours after onset of chest pain where the patient is in the later stages of MI.

In a second embodiment, membrane 18 may have a layer of captured antibody 124 and a corresponding reagent 126. Similarly for each other marker to be detected, a corresponding pair of antibodies and reagents are provided, i.e. 128 and 130, 132 and 134 and control pair 136 and 138. In use, the sample is dropped onto each pair and the results are read in the same manner as described above.

The dry chemistry membrane 118 can be supported by absorbent material 120. Absorbent material 120 will enhance the draw of the serum through the membrane.

A further embodiment for the test kit is to use a blood sample tube which is commonly used to draw blood samples from patients. The inside wall of the tube could act as a carrier for the monoclonal and polyclonal antibodies and reagents. After the sample is drawn from the patient, the user simply shakes the tube so that the antibody reacts with the blood. Colour changes as described above will take place if the cardiac protein is present in the blood.

Although the disclosure describes and illustrates preferred embodiments of the invention, it is to be understood that the invention is not limited to these particular

embodiments. Many variations and modifications will now occur to those skilled in the art. For a definition of the invention, reference is to be made to the appended claims.

CLAIMS

1. A diagnostic kit for detecting a myocardial infarction at early onset of patient chest pain, comprising:

i) a receptacle for receiving and retaining a sample of blood or serum of the patient, and

(ii) a detection means associated with the receptacle and comprising:

a) three or more monoclonal or polyclonal antibodies supported on a solid carrier, each antibody being complementary to a different protein released by the heart muscle during early stages of a myocardial infarction and contactable by the sample, and

b) the necessary reagents independently responsive to each antibody when reacting with its complementary protein, and which collectively provide a response indicative of the diagnostic cardiac condition of the patient.

2. A diagnostic kit according to claim 1 wherein said receptacle and said detection means form an integral structure containing said immobilised antibodies and said reagents in dry chemical form.

3. A diagnostic kit according to claim 1 or 2 wherein the said antibodies are complementary to at least three of the following proteins or enzymes: creatine kinase, myoglobin, myosin light chains, troponin, troponin-I, troponin C, troponin-T and sarcolemmal membrane proteins, triose P isomerase or any low molecular weight cardiac proteins having the characteristics and properties of creatine kinase,

myoglobin or myosin light chains, tropomyosin or any heavy molecular weight cardiac proteins having the characteristics and properties of creatine kinase, myoglobin or myosin light chains, wherein at least two are selected from creatine kinase, myoglobin, myosin light chains and troponin-T.

4. A diagnostic kit according to claim 3 wherein said immobilised antibodies are layered with the corresponding reagent comprising an antibody-enzyme conjugate directed against a different epitope than that recognized by the antibody.

5. A diagnostic kit according to any one of claims 1 to 4, wherein said reagents change colour in response to each antibody reacting with the complementary protein.

6. A diagnostic kit according to claim 2 which is in card form comprising a front panel having a sample window for receiving the sample and a display window for displaying the reagents, a back panel and sealing means for securing the front panel to the back panel sandwiching the detection therebetween thereby to form said integral unit.

7. A diagnostic kit according to claim 6, wherein the carrier for the immobilised antibodies is a dry chemistry membrane.

8. A diagnostic kit according to claim 7, wherein said membrane is supported by an absorbent material to enhance the drawing of the sample to the detection means.

9. A diagnostic kit according to claim 8, wherein said membrane extends over the sample window to the display window and the antibodies and corresponding reagents are in spaced relation from the sample window in the display window.

10. A diagnostic kit according to any one of claims 6 to 9, wherein said front panel is marked to identify a location of the protein antibody reaction.

11. A diagnostic kit according to claim 10, wherein said front panel is further provided with a reflectometer to quantify the concentration of the protein in the sample.

12. A diagnostic kit according to any one of claims 1 to 11, wherein said detection means also includes a control antibody which is complementary with a protein normally found in the serum, and a corresponding reagent responsive to the control antibody reacting with the complementary protein, whereby such response indicates that the test is functioning.

13. A diagnostic kit according to claim 12, as dependent upon claim 6, wherein said control antibody and corresponding protein are spaced on the carrier furthest from the sample window to indicate substantial completion of the test.

14. A diagnostic kit according to claim 2, wherein said receptacle is a sealable clear container and the said detection means is provided on a side wall of the container.

15. A diagnostic kit according to any one of claims 1 to 14, wherein the said antibodies are complementary to creatine kinase and myoglobin.

16. A diagnostic kit according to any one of claims 1 to 14, wherein said antibodies are complementary to creatine kinase, myoglobin and myosin light chain.

17. A diagnostic kit according to any one of claims 1 to 14, wherein said antibodies are complementary to creatine kinase, myoglobin and troponin-T.

18. A diagnostic kit according to any one of claims 1 to 17, which is sensitive to marker concentrations from .5ng/ml to 25ng/ml using less than 100ul of sample with a within run and between run precision coefficient of variation of less than 15%.

19. A diagnostic kit according to claim 1, substantially as hereinbefore described with reference to the accompanying drawings.

Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

Application number
9111965.1

Relevant Technical fields

(i) UK Cl (Edition K) G1B(BAD,BAE,BAG)

(ii) Int Cl (Edition 5) G01N

Search Examiner
MS N R CURTIS

Databases (see over)

(i) UK Patent Office

(ii) Dialog: WPI; Biotech

Date of Search
18 JULY 1991

Documents considered relevant following a search in respect of claims 1-19

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
E	WO 91/01498 A (VIOCLONE BIOLOGICALS INC) - see particularly "Summary of the Invention"	1,2,5-14, 15
X	European Heart Journal, Vol 8, 1987, pages 989-994 - Hoberg et al (see introduction)	1,2,5-16
X	Scan J Clin Lab Invest, Vol 44, 1984, pages 679-682 - Baadsgaard & Schmidt (see introduction)	1,2,5-15

Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family, corresponding document.

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).